DEVICE

Semipermeable Membrane Diffusion Assay

Results

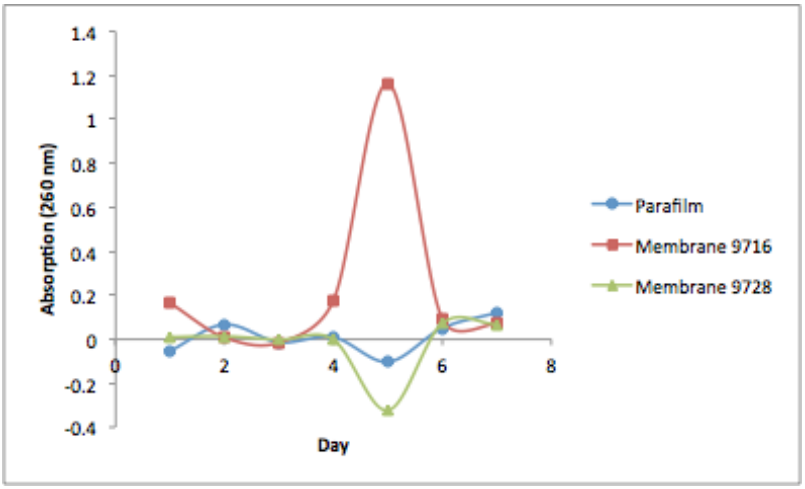

Figure 1. The growth curve of *Bacillus subtilis* with different membranes using absorption at 260 nm over a period of time

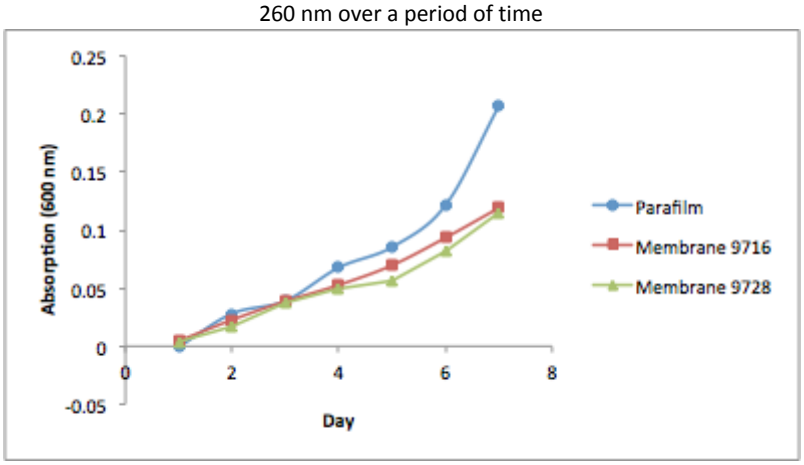

Figure 2. The growth curve of *Bacillus subtilis* with different membranes using absorption at 600 nm over a period of time

Interpretation

This assay was performed to determine whether the membranes used in our transdermal device would be effective in preventing bacterial cells from diffusing through. In running this diffusion assay, our data analysis showed that there was an increased amount of cell detection, meaning that the bacterial cells may or may not be diffusing through. As a result, we were not able to conclude that our membranes would be effective in acting as a filter.

Project Achievements

This does not produce any tangible benefit for our project, and another approach must be tried

Future Plans

The experiment should be repeated under aseptic conditions with a flame to ensure that there are no other sources of contamination. Also, plating the samples on chloramphenicol or hygromycin plates and adding chloramphenicol or hygromycin to the saline solution will allow us to determine whether the bacteria passing through the membrane was the one we used in our experiment. Since we got positive readings in our negative control, we decided that a better negative control would be capping off the falcon tube rather than using parafilm.

Filter Sterilization Membrane Diffusion Assay

Results

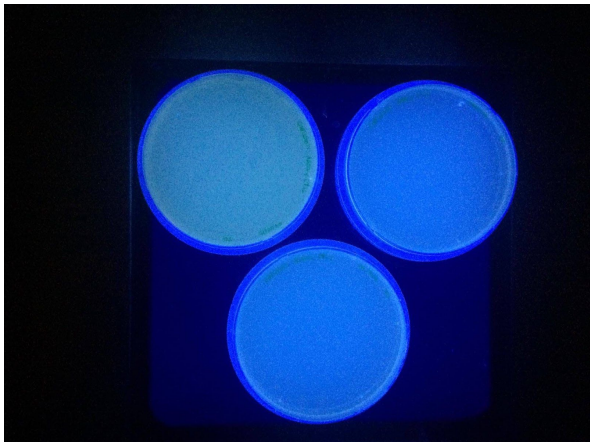

Fig 3. The left plate is the positive control using overnight cultures and without a syringe filter, the right plate is the negative control using LB media and syringe filter, and the bottom the test plate with the membrane.

Interpretation

From the results of the overnight plates, it can be concluded that the 0.2 micron syringe filter prevented the diffusion of bacteria from the syringe into the surrounding environment as no overnight cultures grew on the test plates. These were confirmed with the positive plates growing overnight cultures meaning our plates were not defective and our negative plates meaning there was no contamination.

Project Achievements

From these results, we were able to find a suitable semipermeable membrane that prevented the diffusion of bacteria cells into the

surrounding environments. This helped with the applied design by creating a functional prototype.

Future Plans

The next time would be to determine if BBI can diffuse through this membrane and find a method to manufacture the 0.2 micron syringe filter for larger application in our patch design.

Backing Layer Growth Curve Assay

Results

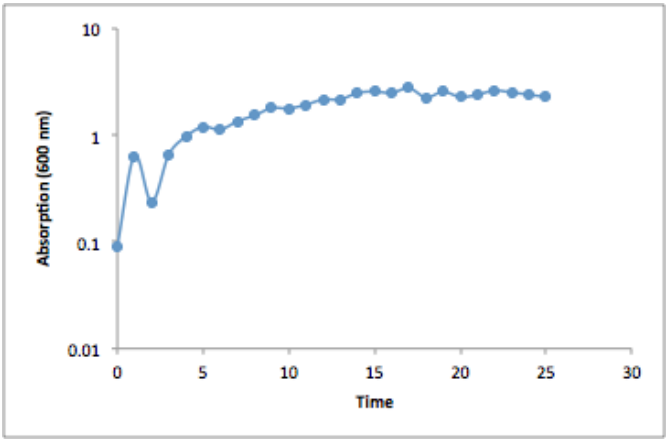

Figure 4. The growth curve of *Bacillus subtilis* with limited oxygen by a backing layer using absorption at 600 nm over a period of time

Interpretation

Following chassis protocol, we are performing growth curves with the backing layer to determine if limiting the gas exchange using the backing layer will affect cell growth. The data from our experiment showed a growth curve that is similar to those found by the chassis group. Therefore, the backing layer will be a suitable material in our transdermal device since it will not limit the growth of our bacteria cells.

Project Achievements

Determined that the backing layer did not prevent cell growth and were able to find a suitable membrane for our patch design.

Future Plans

Following the first backing layer growth curve assay, we consulted our mentors and determined that the assay was not effective in determining whether our backing layer would be a limiting factor for gas exchange. This is due to the fact that there is 10x the volume of oxygen in the culture tube to begin with. Therefore, the rate of gas exchange through the backing layer will be negligible. The assay

should be repeated with a different protocol to determine whether the bacterial cells can be starved of oxygen with our backing layer. In this assay we should apply the backing layer on top tightly to prevent excess oxygen in the patch. We would then put it in the shaker at 35°C + 10 rpm and take readings at day one, three and seven. This way, we will be able to gain insight on the lifetime of our patch as well.

Backing Layer Survival Assay

Results

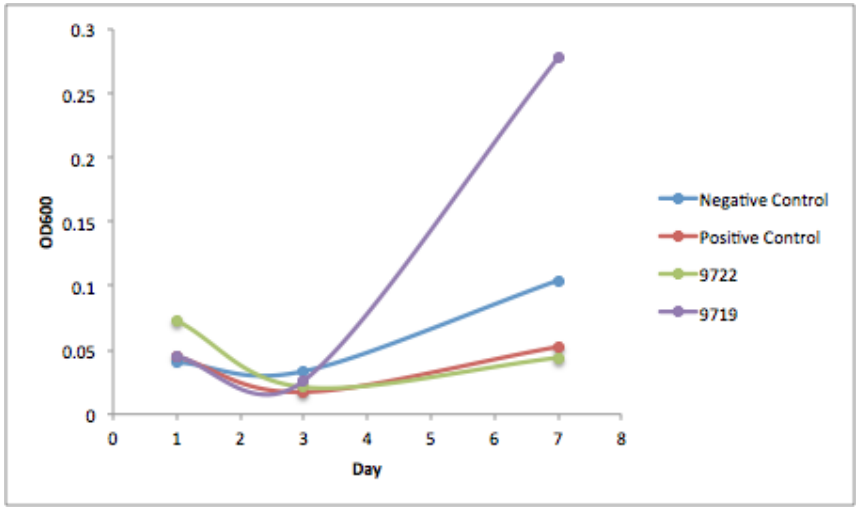

Figure 5. The optical density of *Bacillus subtilis* over a 7 day period of time using different backing layer materials

Interpretation

This assay was done to determine whether the bacterial cells can be starved of oxygen with our backing layer. We can show that from day 1 to 3 there is is similar growth in the 9719, negative and positive controls. 922 had a higher initial growth rate, however this is not what we expect for a seven day trend. Our data suggests that the cells that grew, died, and then regenerated by day 7. However, there was no change in media which may mean the cell counts/OD readings on day 7 were due to dead cells.

Project Achievements

We can conclude that the backing layer has no real effect on cell growth

Future Plans

Future plans entail that we must find out if the backing layer has any detrimental effects whatsoever on cells. This may be an increased mutagenesis or whether or not the backing layer can support cells for

more than 7 days.